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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We developed a sensor for the detection of microbial and molecular biological warfare agents. The completed sensor consists of a semiconducting nanocrystal quantum dot covalently linked to the 5' end of a pathogen-specific aptamer. The second portion of the sensor is an oligomer strand that is complementary to the aptamer and has a gold nanoparticle covalently bound to its 3' terminus. In testing the sensor against pathogen stimulants, it was found that molecular targets provided great reproducibility and selectivity. After optimizing the sensor parameters, detection limits for molecular stimulants were found to be at the nanomolar range, even in the presence of interfering species. On the other hand, microbial stimulants showed much less selectivity when tested against control spores. This was due, in part, to the inadequate on/off signaling of a solid substrate as opposed to the solution target described above. Similar behavior was observed (false positives) when testing against other biological entities such as E.coli and fungus. As well, it was found that the physiological state of the test spores strongly controls selectivity, i.e., spore state, vegetative state, or somewhere between, and should be the subject of future studies.

15. SUBJECT TERMS

biosensor, biological warfare agent, aptamers, nanoparticles

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Quantum Dot Nanocrystals Coupled to DNA Aptamer Sensors for Biological Weapons Detection

AFSOR FA9550-06-1-0365

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EXECUTIVE SUMMARY OF WORK COMPLETED:

BioNanoSensor Design.

We developed a new method for detecting biological pathogens using a nanoparticle-aptamer-based sensor. The bionanosensor was designed, optimized, and tested for robustness against stimulants of molecular and microbial biotoxins, e.g., thrombin and bacillus thuringiensis (BT) spores. The bionanosensor is based on specially modified luminescent nanocrystal quantum dots (QDs) as the reporter, and DNA aptamers as the selector, to comprise a sensor for recognition of biological toxins. In its initial state, the QD luminescence is deactivated by a quencher moiety, a gold nanoparticle (GNP) attached to a DNA oligomer complementary to the aptamer. In the presence of the target microbial toxin, the complementary oligomer is displaced, releasing the GNP quencher—activating QD luminescence.

Pathogen-specific DNA aptamer sequences developed by researchers at Brooks Air Force Base and others were utilized to specifically bind biotoxins according to unique ligand binding features of the aptamer against its target. As shown in Fig. 1, the 5' terminus of the aptamer will be bound a highly luminescent QD of a specific emission wavelength indicative of the pathogen target. A complementary oligomer strand the aptamer will have a gold nanoparticle (GNP) on its terminus that is adjacent to the QD. This duplex will not emit light due to the quenching of QD luminescence by the GNP. However, in the presence of a biotoxin, the binding affinity of the aptamer to the pathogen will result in displacement of complementary oligomer bearing the GNP quencher, which will activate the luminescence of the QD, thus providing a signal that a biotoxin has been encountered. Importantly, the tunable feature of QD emission has the potential to allow for simultaneous detection of a multitude of various pathogenic targets in infrared or visible regions. Excitation and collected emission of the QD was accomplished in a variety of case-specific ways from fluorescence/Raman microscopy and spectroscopy to atomic force and near-field optical microscopy and electrophoresis.

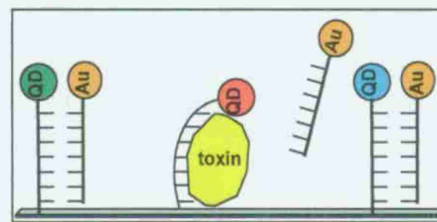


Fig. 1 Illustration of pathogen sensor.

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Nanobiosensor Integrity.

Considerable effort also focused on developing a robust bionanosensor that could maintain its selectivity and detectability in various environmental conditions. Although QD luminescence provides a much greater detection capability as compared to other nanoparticles, QD photoluminescence can also be easily compromised when exposed to various environmental parameters such as temperature, pH, oxygen content, and long-term solution stability. An explicit statement of collaborators at Brooks Air Force Base was that for QDs to be effective for battle-field or civilian applications, it must be a resilient design capable of functioning under a wide range of environmental conditions. To address this issue, we developed a method for encapsulating the signaling agent (QDs) within a protective silica shell. An example is shown in Figure 2. It was found that encapsulating QDs in this way preserved photoluminescence, provided a greater degree of flexibility in surface functionalization and biocompatibility, and was less toxic than non-encapsulated QDs. As shown in the image, high-level monodispersity of the preliminary methods are easily prepared, and early experiments indicate the encapsulated product is viable for extended periods of time (months), whereas 'traditional' derivatization without a protective barrier may last for only a few assays. It is imperative that the QDs exhibit a relatively long use-life if they are to be suitable for environmental monitoring of biological pathogens.



Fig.2 Silica-encapsulated quantum dots. The dark features represent the quantum dot nanoparticle and the hazy portion is the silica shell.

Quality Control.

Moreover, since beginning work with QDs, it has come to our attention that there is an often overlooked need for improvement in quality control for the efficacy of QD surface modifications and sensor and bioconjugation, i.e., monitoring reaction efficiencies, completeness, and product stability. By monitoring the stepwise surface modifications and subsequent bioconjugations using a combination of chromatographic, electrophoretic, spectroscopic, and microscopic techniques, we were able to have more control over manipulation of QD-aptamer and GNP-complement binding. It was through these studies that we could quantitatively determine the optimal DNA:nanoparticle binding.

BioNanoSensor Testing: Molecular Biotoxin Simulant.

An example for colorimetric and photoluminescence detection of the is shown in Figure 3 using the molecular biotoxin stimulant, thrombin, was readily detectable in solution using our bionanosensor design. High selectivity was observed compared to controls and in the presence of competitive binding species. Good detection limits (nanomolar) were also observed. In addition, a colorimetric response was observed, allowing the sensor design to take advantage of lost-cost colorimetric detection as well as highly-sensitive photoluminescence detection. Also shown in Figure 3 was part of our biggest challenge. As can be seen, the background photoluminescence signal is substantial in the "off" state and could be further optimized. Therefore, considerable effort was given to optimizing and selecting GNP quenching agents that best provided luminescence quenching. As well, efforts were made to optimize the ratio of GNPs per QD complex. Thus far, it has been found that the optimal gold diameter is 13-15 nm and the optimal GNP:QD quenching ratio was 2:1. Nonetheless, results were only mildly improved, which indicated further research needs to be done on characterizing the nanoparticle quenching and energy transfer mechanisms of the sensor.

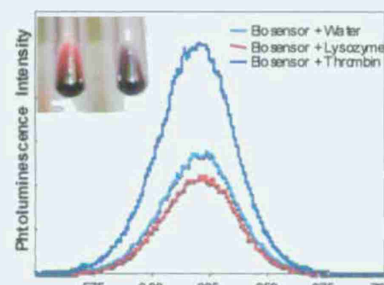


Fig.3 Photoluminescent and colorimetric biosensor response in the presence of the protein toxin simulant.

BioNanoSensor Testing: Microbial Biotoxin Simulant.

Tests were conducted using *Bacillus thuringiensis* (BT) spores as a pathogenic simulant. The QD-aptamer biosensor readily illuminated sections of the spore sample as the spores progressed into

a vegetative state. Although encouraging, a control simulant, *Bacillus globigii* (BG) also showed a dynamic response to the QD biosensor. We believe that further characterization of the QD biosensor interaction with the spore is needed to determine if there is a growth phase dependence on the recognition efficiency of the current aptamer. Similarly, lack of specificity was observed when testing against *E. coli* and fungus.

Listing of Major Accomplishments and Findings:

- Developed capillary electrophoresis (CE) methods for the separation of quantum dot (QD) nanoparticles and various derivatives for incorporation into complex matrices
- Characterized QD ligand exchange model for dynamic mixed ligand exchange through new CE methods
- Refined CdSe, CdSe/ZnS, and CdSe/ZnSe/ZnS QD synthesis and purification strategies
- Furthered development of silica encapsulation of QDs to enhance:
 - monodispersity
 - diverse functionality
 - solution stability
 - thin shell size
- Performed test studies with silica-encapsulated QDs
 - cytotoxicity
 - antibody/antigen immunoassay
 - aptamer binding
 - gold nanoparticle (GNP) quenching
- Continued studies with QD-aptamer detection of molecular bioweapon stimulant, thrombin and the spore bioweapon stimulant, *Bacillus thuringiensis* (BT)
 - refined procedure for binding aptamer to QDs
 - dynamic response in solution for thrombin
 - colorimetric and fluorogenic confirmation of target detection
 - time-course studies conducted
 - optimal quenching with 13 nm GNPs
 - QD-aptamer specificity measured relative to control *Bacillus globigii* (BG), *E. coli*, and fungus.
- Refined synthesis and handling procedures for various GNP surfaces
- Optimized addressed grids for microscopy & spectroscopy applications
- Demonstrated multidimensional analysis of addressed grids for microscopy characterization using various surface probing techniques
- Single-nanoparticle spectroscopy vis-NIR (400-1600nm)
- Initial adsorption spectra of adsorbed chromophores on GNPs

Personnel Supported:

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Kenneth Roberts, Associate Professor, P.I.
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Daniel Stanley, PhD graduate student
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Danny Wasielewski, undergraduate

Publications

- Dahanayaka, D. H.; Bayer, I. S.; Biswas, A.; Roberts, K. P.; Bumm, L. A., Nanotechnology: Quantum Dot Technology for Advanced Biomedical Applications. *Vacuum Technology and Coating* **2009**, 10 (3), 26-32.
- Near-field spectroscopy of surface plasmons in flat gold nanoparticles, M. Achermann, K. L. Shuford, G. C. Schatz, D. H. Dahanayaka, L. A. Bumm, V. I. Klimov, *Optics Letters*, **32**(15), 2254-2256 (01 Aug 2007).
- Fluorescent In Situ Hybridization Employing the Conventional NBT/BCIP Chromogenic Stain, L. A. Trinh, M. D. McCutchen, M. Bonner-Fraser, S. E. Fraser, L. A. Bumm, and D. W. McCauley, *BioTechniques* **42**(6), 756-759 (Jun 2007).
- Characterization of CdSe Nanocrystals and their Interaction with Micellar Aggregates by Means of Capillary Electrophoretic Techniques, Oszwaldowski, S.O., Vasicek, L., Tonev, A., and Roberts, K.P., *Nanotech 2007*, **2007**, 4, 332.
- Characterizations in Semiconductor Nanocrystal Sensors, Roberts, K.P., Swenson, K., Grigsby, L., Chambers, H., Wiser, R., Oszwaldowski, S.O., Curtis, M.E., Johnson, M.J., Bumm L., ISA, 2007, 207A, 459 (October 2007).
- Characterization of surfactant-coated CdSe nanocrystals by means of spectroscopic and capillary electrophoretic methods, Oszwaldowski, S., Grigsby, L.K., and Roberts, K.P., *Analytica Chimica Acta*, **2008**, (in revision).
- Single Flat Gold Nanoparticle Microscopy and Spectroscopy, W.D. Tennyson, C.E. Allen, D.R. Freno, D.H. Dahanayaka, L.A. Bumm, *Microscopy and Microanalysis*, (2008) (in press).

Dissertations (five Master's theses)

1. **Brian L. Walker**, *Optimized binding of DNA aptamers to nanoparticles for biosensing applications*, May 2010. Thesis Location: University of Tulsa Library
2. **Holly A. Chambers**, *A quantum dot-aptamer beacon sensor coupled with gold nanoparticles for the fluorescent and colorimetric sensing of target bioweapons*, May 2008. Location University of Tulsa Library
3. **Laura K. Grigsby**, *Versatile silica encapsulated quantum dots for bio and photonics applications*, May 2008. Thesis Location: University of Tulsa Library
4. **John W. Colson**, *Surface Enhanced Optical Absorption? A study of optical absorption by dyes adsorbed on gold nanoparticles*, senior thesis in chemistry, May 2009. Thesis Location: University of Oklahoma Library
5. **Matthew W. Fenwick**, *Instrumentation and Software for Single-particle Light-scattering Spectra of Nanoparticles*, senior thesis in chemistry, May 2009. Thesis Location: University of Oklahoma Library

Patents

none

Presentations (from June 2006 to June 2010)

Summary: 5 invited; 3 at national meetings; 13 at regional meetings

Invited Papers, Colloquia, and Seminars Presented

- (KPR) **Nanoparticle Biosensors**, Oklahoma Nanotechnology Initiative--NanoFocus, Oklahoma City, OK, The University of Oklahoma, 6-7 Mar 2008.
- (KPR) **Biosensing with NanoParticles**, Oklahoma Microscopy Society, The University of Oklahoma, Norman, OK, May 2008.
- (KPR) **Advancing in Academia**, Oklahoma Medical Research Foundation, Oklahoma City, OK, May 2008.
- (LAB) **Flat Gold Nanoparticles: Atomically-Flat Substrates for Surface Science and Molecular Plasmonics**, C-PCS Seminar, Los Alamos National Laboratory, Los Alamos, NM, 10 Aug 2007.
- (LAB) **Flat Gold Nanoparticles: Atomically-Flat Substrates for Surface Science and Molecular Plasmonics**, Physics Colloquium, The University of Alabama, Tuscaloosa, AL, 31 Oct 2007.

Contributed Papers Presented

Key: G=graduate student presentation; U=undergraduate student presentation; C=collaborator;
O=oral; P=poster

National Meetings

- (GP) **Quantum Dot—Aptamer Conjugates as Bioweapon Sensors**, Pittsburg Conference, New Orleans, LA (3-6 Mar 2008). Presented by Holly Chambers.

(GO) **Surface preparation of supported flat gold nanoparticles for use as Au{111} substrates**, ACS Colloid and Surface Science Symposium, University of Delaware, Newark, DE (24-27 Jun 2007). Presented by D.H. Dahanayaka.

(GP) **Single-Nanoparticle Light-Scattering Spectra of Flat Gold Nanoparticles (FGNPs): A Study of the Effect of Nanoparticle Treatment**, AVS, Seattle, WA (14-19 Oct 2007). Presented by W.D. Tennyson.

Regional Meetings

(GO) **Microscopy & Single-Nanoparticle Spectroscopy of Flat Gold Nanoparticles: A Study of the Effect of Nanoparticle Treatment**, OAS/OMS, Tulsa Community College, OK (02 Nov 2007). Presented by W.D. Tennyson.

